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REVIEW ARTICLE

Serine proteinases in the turnover of the cartilage extracellular matrix in the joint: implications for therapeutics

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Cartilage destruction is a key characteristic of arthritic disease, a process now widely established to be mediated by metzincins such as MMPs. Despite showing promise in preclinical trials during the 1990s, MMP inhibitors for the blockade of extracellular matrix turnover in the treatment of cancer and arthritis failed clinically, primarily due to poor selectivity for target MMPs. In recent years, roles for serine proteinases in the proteolytic cascades leading to cartilage destruction have become increasingly apparent, renewing interest in the potential for new therapeutic strategies that utilize pharmacological inhibitors against this class of proteinases. Herein, we describe key serine proteinases with likely importance in arthritic disease and highlight recent advances in this field.

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Abbreviations

ADAMTS, a disintegrin and metalloproteinase with thrombin motifs; APC, activated protein C; CIA, collagen-induced arthritis; CTSG, cathepsin G; DMM, destabilization of the medial meniscus; ECM, extracellular matrix; HtrA, high temperature requirement proteinase A; MAC, membrane attack complex; NE, neutrophil elastase; NSP, neutrophil serine proteinases; OA, osteoarthritis; PAR, proteinase-activated receptor; PCSK, proprotein convertases subtilisin and kexin type; PR3, proteinase-3; RA, rheumatoid arthritis; RCL, reactive centre loop; SERPIN, serine proteinase inhibitor; TIMP, tissue inhibitor of metalloproteinase; tPA, tissue-type plasminogen activator; TTSP, type II transmembrane serine proteinases; uPA, urokinase-type plasminogen activator

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Introduction

The word *arthritis* comes from the Greek word *arthron* – meaning joint – and *itis* meaning inflammation. Whilst a relatively heterogeneous group of diseases, arthritic diseases typically involve pain, immobility and destruction of the extracellular matrices of the synovial joints. The most prevalent (and most studied) are osteoarthritis (OA) and rheumatoid arthritis (RA). OA has an insidious onset and typically affects older people. Originally believed to be a disease of 'wear-and-tear', it is now considered a result of distinct molecular pathways that manifest as gradual destruction of the articular cartilage, osteophyte formation, subchondral bone thickening (sclerosis) and often a degree of synovial inflammation. RA is an autoimmune disease with rapid onset, driven by a highly inflammatory and hyperplastic synovial membrane which invades the joint. As a further level of complexity, many RA patients present with 'secondary' OA, including osteophyte development which further contributes to joint impairment (Figueiredo *et al.*, 2016). Importantly, although considered separate diseases clinically, RA and OA both result in the destruction of articular cartilage and exposure of the underlying bone.

Cartilage is a unique tissue consisting of a rich extracellular matrix (ECM), devoid of vascularization or innervation, harbouring a single cell type, the chondrocyte. Cartilage ECM is composed predominantly of type II collagen, which provides tensile strength whilst the large proteoglycan aggregate, aggrecan, provides compressive strength by drawing water from the surrounding area. Cartilage also contains numerous less abundant matrix components such as biglycan, cartilage oligomeric matrix protein, decorin and fibronectin.

Metalloproteinases are the class of proteinase attributed as the key effector proteinases in cartilage destruction. MMPs, in particular the collagenases (**MMP1**, **MMP8** and **MMP13**), are responsible for the destruction of cartilage collagen which, due to slow synthesis and repair processes, is essentially irreversible (Rowan *et al.*, 2008). Whilst MMPs can also cleave aggrecan, a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) proteinases – specifically the aggrecanases (**ADAMTS4**, **ADAMTS5**) – are suggested to be the primary mediators for cleaving this ECM component. Despite promising preclinical results, MMP inhibitors have proven overwhelmingly disappointing clinically. Subjects exhibited a range of off-target effects, including a 'musculoskeletal syndrome' manifesting as arthralgia, immobility and contractures (Fingleton, 2008). The failure of MMP inhibitors was largely due to a lack of specificity since they targeted the catalytic site (Rowan *et al.*, 2008) which is highly conserved amongst MMPs. Moreover, it is now established that many MMPs function as 'anti-targets' and hence the specificity of inhibitors to target particular MMPs is crucial.

Serine proteinases are typically extracellular proteinases, which combined with metalloproteinases, represent almost 2/3 of all proteinases (33% metallo, 32% serine; Ugalde *et al.*, 2010). They utilize a catalytic triad of serine, histidine and aspartate which provides an optimal electrostatic environment for nucleophilic attack of a carbonyl group on a peptide backbone from the hydroxyl group of the catalytic serine. Important physiological roles for serine proteinases include blood coagulation, digestion, fertility and wound healing. More recently, genomic advances have led to the discovery of new serine proteinases and indeed entirely new sub-families. Our understanding of the roles of serine proteinases in arthritis pathogenesis has been vastly improved by the use of *in vivo* models, of which there are many (reviewed in Vincent *et al.*, 2012). Table 1 outlines key findings from such models. Although proteolysis of cartilage in arthritis is predominantly metalloproteinase-driven, serine proteinases perform crucial functions such as proMMP activation, direct ECM degradation, cytokine regulation and receptor activation. Herein, we discuss evidence for the involvement of serine proteinases associated with the plasminogen-plasmin system, immune cells, complement, type II transmembrane serine proteinases (TTSP), high temperature requirement proteinases and proprotein convertases. Furthermore, we highlight recent advances in our understanding of the roles of endogenous serine proteinases and **proteinase-activated receptors (PARs)** in cartilage breakdown in arthritis. Improving our understanding of this enzyme class should identify more tractable pharmacological targets to prevent the cartilage destruction that characterizes arthritis.

Serine proteinases and cartilage ECM turnover

Plasminogen/plasminogen activators

Plasminogen is processed into its active form, **plasmin**, by the plasminogen activators (PAs) urokinase-type and tissue-type plasminogen activator (**uPA** and **tPA**, respectively), which are themselves synthesized as pro-enzymes. Plasmin has broad substrate specificity and therefore has numerous functions although it is perhaps best described in fibrinolysis, a process essential during blood clotting (Draxler and Medcalf, 2015). The presence of key components of the plasminogen/PA systems has been examined in the joint, and whilst the expression of plasminogen mRNA is undetectable in articular cartilage (Milner *et al.*, 2010), the proteinase has been detected in synovial fluid (Caughey and Highton, 1967). uPA has also been detected in synovial fluids of both OA and RA patients, with levels significantly elevated in RA (Busso *et al.*, 1997).

Plasmin can degrade the ECM directly by cleavage of components such as **fibronectin**, glycoproteins and proteoglycans, but it can also activate numerous proMMPs (Milner *et al.*, 2008). In cartilage explant culture, a potent uPA inhibitor protected cytokine-stimulated cartilage from collagen release, although the selectivity of the inhibitor means off-target inhibition cannot be excluded (Milner *et al.*, 2001). The addition of plasminogen to this system induces collagen release early, suggesting the presence of active plasminogen activators, conversion to plasmin and subsequent proMMP activation (Milner *et al.*, 2001).

Different animal models of arthritis have yielded conflicting results using transgenic mice deficient for plasminogen/PA system components, likely due to differences in disease initiation and progression between models. Plasminogen- and uPA-deficient mice both exhibited

Table 1

Summary of key studies involving the effect of serine proteinase gene deficiency or inhibition in animal models of arthritis

Serine proteinase/ proteinase substrate	Treatment	Genotype	Model	Effects	Reference
CTSG NE	–	CTSG$^{-/-}$ (*Ctsg*) NE$^{-/-}$ (*Elane*)	Arthritis induced by anti-type II collagen antibody	• Clinical arthritis with low mean arthritis score. • Fewer inflammatory cells in subsynovial tissue space, less exudate and fibrin-like deposition in joint space. • Some preservation of proteoglycan content.	(Adkison *et al.*, 2002)
		CTSG$^{-/-}$ x NE$^{-/-}$		• Clinically resistant to arthritis induction. • Little presence of inflammatory cells in subsynovial space. • Little or no exudate in joint space and no proteoglycan loss.	
	NE Inhibitor (orally)	WT	CIA	• Less severity in joint swelling. • Inhibition of cartilage and bone destruction, as well as pannus formation.	(Janusz and Durham, 1997)
	NE Inhibitor (i.p.)	WT		• Reduced disease incidence and severity, leukocyte infiltration in the joint cavity, pannus formation and cartilage destruction.	(Kakimoto *et al.*, 1995)
Furin	Recombinant protein (i.p.)	WT	CIA	• Reduced arthritis score, synovial pannus thickness invading the joint, cartilage and bone destruction, MMP expression and activity, and local concentration of pro-inflammatory cytokines. • Increased local concentration of anti-inflammatory cytokines.	(Lin *et al.*, 2012)
	Inhibitor (i.p.)			• Increased arthritis score, synovial pannus thickness and invasive into the joint, MMP expression and local concentration of pro-inflammatory cytokines. • Reduced local concentration of anti-inflammatory cytokines. • No effect on bone loss.	
Granzyme A (GzmA)	–	GzmA$^{-/-}$ (*Gzma*)	CIA	• Reduced clinical scores, pro-inflammatory cytokine levels in serum, and number of osteoclasts. • Almost normal joints with mild pannus formation and absence of bone and cartilage erosions.	(Santiago *et al.*, 2017)
Matriptase	Inhibitor (IA)	WT	DMM	• Reduced cartilage damage. • Less antibody immunostaining of ADAMTS-cleaved and MMP-cleaved aggrecan, and	(Wilkinson *et al.*, 2017b)

continues

Table 1

(Continued)

Serine proteinase/ proteinase substrate	Treatment	Genotype	Model	Effects	Reference
				collagenase-cleaved type-II collagen at damage sites.	
Protease-activated receptor 2 (PAR2)	–	PAR2$^{-/-}$ (*F2rl1*)	DMM	• Delayed osteophyte maturation. • Altered weight bearing on affected joints suggesting reduced pain perception.	(Huesa *et al.*, 2016)
				• Lower cartilage damage and osteosclerosis scores.	(Ferrell *et al.*, 2010)
			ACL	• Lower levels of cartilage damage.	
	Inhibitor (i.p.)	WT	DMM	• Reduced OA pathology. • Lower cartilage damage and osteosclerosis scores.	
SERPINA1 (α1-AT)	Recombinant protein (i.p.)	WT	CIA	• Delay in arthritis onset, amelioration of disease progression and reduced incidence of severe arthritis.	(Grimstein *et al.*, 2011)
SERPINE1 (PAI-1)	–	PAI-1$^{-/-}$ (*Serpine1*)	AIA	• Reduced inflammatory response, synovial infiltration, proteoglycan loss and fibrin levels. • Increased activity of plasminogen activator and fibrinolysis.	(Van Ness *et al.*, 2002)
Tryptase	Inhibitor (IA)	WT	mBSA/ IL-1β (IA)	• Reduced tryptase-like activity, oedema formation, and cytokine production. • No difference in neutrophil infiltration, hyaline cartilage degeneration and subchondral bone erosion.	(Denadai-Souza *et al.*, 2017)
Plasminogen (Plg)	–	Plg$^{-/-}$ (*Plg*)	AIA	• Comparable levels of arthritis at early time-points with wild-type, but exacerbated scores at later stages.	(Li *et al.*, 2005a)
				• Prolonged joint inflammation and synovial thickness. • Increased bone erosion and fibrin accumulation.	(Busso *et al.*, 1998)
			CIA	• None of the mice developed clinical signs of inflammation. • Normal joint morphology without inflammation and tissue destruction in the peripheral synovial tissues. • No infiltration of neutrophils and only a few resting macrophages. • Following IA injection of saline or CII (LIA), mice developed arthritis.	(Li *et al.*, 2005a, Li *et al.*, 2005b)

continues

Table 1

(Continued)

Serine proteinase/ proteinase substrate	Treatment	Genotype	Model	Effects	Reference
			CAIA	• No signs of inflammation or tissue destruction. • After i.v. injection of plasminogen, mice developed active inflammation and tissue destruction.	
Urokinase plasminogen activator (uPA)	–	uPA-1$^{-/-}$ (*Plau*)	AIA	• Joint inflammation. • Increased synovial thickness, and synovial fibrosis in some mice. • Reduced proteoglycan content. • More pronounced bone erosion and fibrin accumulation.	(Busso *et al.*, 1998) (De Nardo *et al.*, 2010)
			CIA	• Reduced severity and incidence of arthritis. • Delayed disease onset and lower clinical scores.	(Li *et al.*, 2005b)
				• 75% mice developed less severe CIA. • Reduced gene expression of TNF, IL-1β, IL-6, MCP-1, t-PA, u-PA, u-PAR, MMP3, MMP9, MMP13 and ADAMTS4. • No changes in ADAMTS5 mRNA levels.	(Cook *et al.*, 2010)
			CAIA	• 43% of mice developed only mild arthritis.	
			K/BxN	• 60% of mice developed only mild arthritis. • Relatively normal joints with minimal differences in arthritis development. • Following IA injection of saline, mice developed arthritis (increased joint swelling, cellular infiltration, bone erosion and fibrin(ogen) staining and reduced proteoglycan content).	(De Nardo *et al.*, 2010)

ACL, anterior cruciate ligament transection; AIA, antigen-induced arthritis; CAIA, CII antibody-induced arthritis; IA, intraarticular; LIA, local injection-induced arthritis; mBSA/IL-1β, methylated BSA/IL-1β-induced arthritis; STIA, K/BxN serum transfer-induced arthritis.

protection in collagen-induced arthritis (CIA) and K/BxN models of inflammatory arthritis (Li *et al.*, 2005b; Cook *et al.*, 2010). Conversely, these mice demonstrated marked disease exacerbation following antigen-induced arthritis (Busso *et al.*, 1998; Li *et al.*, 2005a). It is hypothesized that the presence or absence of trauma during arthritis induction is crucial to the effects these fibrinolytic proteinases have on disease development (Li *et al.*, 2005a; De Nardo *et al.*, 2010). More recently, a TNFα-driven murine model of inflammatory arthritis (Keffer *et al.*, 1991) demonstrated that plasminogen-deficient mice exhibited protection from arthritis in the knee joints but exacerbated arthritis development in paw joints further highlighting the importance of the joint micro-environment on disease progression (Raghu *et al.*, 2014).

Type II transmembrane serine proteinases

In OA, cartilage destruction is initially observed in the pericellular matrix surrounding the chondrocyte (Hollander *et al.*, 1995) making membrane-bound serine proteinases likely candidates for a role in disease initiation. Indeed, increased expression of the TTSP **matriptase** (originally called suppressor of tumourigenicity 14) was described in OA cartilage compared to control healthy tissue (Milner *et al.*, 2010). Matriptase has previously been described as a **proMMP3** activator (Jin *et al.*, 2006), a prime enzyme involved in the activation of many other proMMPs. Matriptase was also demonstrated to directly activate proMMP1, a key collagenase involved in cartilage destruction. Interestingly, when added to OA, cartilage in explant culture matriptase induced significant expression of proMMP1 and proMMP3 as well as significant collagen and proteoglycan release, which was dependent on both metalloproteinases and **PAR2**, a well-described substrate for matriptase (Milner *et al.*, 2010; Wilkinson *et al.*, 2017b). Importantly, cartilage protection in a destabilization of the medial meniscus (DMM) model of OA (Glasson *et al.*, 2007) was observed in a dose-dependent manner following administration of matriptase inhibitors (Wilkinson *et al.*, 2017b). Interestingly, since matriptase can auto-activate, it has been postulated as an initiator of proteolytic cascades (Qiu *et al.*, 2007). A related TTSP, hepsin, can activate proMMP1 and proMMP3 and also induce cartilage destruction when added to human OA cartilage cultures. However, the lower levels of collagen release compared to matriptase are likely attributable to the markedly reduced capacity of hepsin for PAR2 activation (Wilkinson *et al.*, 2017a), further highlighting how even related proteinases can have overlapping yet distinct substrate repertoires (Qiu *et al.*, 2007).

Immune cell-derived serine proteinases

Immune cell infiltration is a key driver of arthritis pathology, particularly for inflammatory arthropathies. There are numerous examples of leukocyte-derived serine proteinases which likely contribute towards disease. The neutrophil serine proteinases (NSPs) – **neutrophil elastase** (NE), **cathepsin G** (CTSG) and **proteinase-3** (PR3) – are stored in granules of polymorphonuclear leukocytes (neutrophils, eosinophils and basophils) and released during degranulation. Roles for these proteinases have been particularly well described in inflammatory arthritis such as RA, in which neutrophils play a central role in disease aetiology. Indeed, perhaps unsurprisingly, NE levels are significantly higher in inflammatory arthropathies (Borth *et al.*, 1986; Huet *et al.*, 1992; Elsaid *et al.*, 2003). Mice deficient in **dipeptidylpeptidase**-I (DPP1; also known as cathepsin C), a key cysteine proteinase involved in NSP activation in the lysosomal pathway, are protected against collagen-induced and collagen antibody-induced arthritis (Adkison *et al.*, 2002; Hu and Pham, 2005), with reduced proteoglycan depletion and cellular infiltrate. Similarly, double knockout $NE^{-/-}/CTSG^{-/-}$ mice demonstrated a reduced arthritis score compared to wild-type mice (Adkison *et al.*, 2002), further highlighting the importance of these enzymes in disease progression. Neutrophil degranulation induces potent cartilage destruction (Hilbert *et al.*, 2002) and NE inhibition has also proven effective at reducing articular cartilage destruction in the CIA model of RA in mice (Kakimoto *et al.*, 1995; Janusz and Durham, 1997). Due to the broad specificity of NSPs, their most probable role in RA progression is that of direct ECM destruction. Indeed, NE and CTSG induce potent destruction of cartilage proteoglycan *in vitro* and *in vivo* (McDonnell *et al.*, 1993). NE has also been demonstrated to activate proMMP3 (Nagase *et al.*, 1990) and **proMMP9** (Ferry *et al.*, 1997), the activation of which may also contribute to joint destruction. Interestingly, NE also potently degrades **tissue inhibitor of metalloproteinase (TIMP)1**, further highlighting the interaction of serine and metalloproteinase activities (Nagase *et al.*, 1997). However, more precise, regulatory roles for these proteinases are emerging, including cytokine processing, receptor shedding and apoptosis (Pham, 2008). For example, NE, CTSG and PR3 can disarm PAR2 by cleaving the C-terminal of the canonical activation site. Interestingly, NE can also induce non-canonical, *biased* PAR2 signalling, indicating that NE may play a role in modulating inflammatory responses (Ramachandran *et al.*, 2011). NSPs in OA are less well described. However, inflammation in the pathogenesis of OA is increasingly recognized, at least within a subset of patients. Recently, a PAR2-dependent role for NE in inflammation and pain has been described in a murine OA model induced by monoiodoacetate (Muley *et al.*, 2017). Furthermore, administration of **sivelestat** (a licensed NE inhibitor) reduced structural changes following post-traumatic knee injury in rats (Yu *et al.*, 2017).

Granzymes are serine proteinases stored in the cytoplasmic granules of cytotoxic T-lymphocytes and natural killer cells, which, upon release into the intercellular space (termed the 'immunological synapse'), play essential roles in inducing apoptosis in target cells (Kurschus and Jenne, 2010). **Granzyme A** and **granzyme B** are detectable in RA synovial fluids (Spaeny-Dekking *et al.*, 1998), and levels of the latter correlate with joint erosion in rheumatoid factor positive patients with early RA (Goldbach-Mansky *et al.*, 2005). This proteinase can directly degrade cartilage components, including aggrecan (Froelich *et al.*, 1993; Ronday *et al.*, 2001), and is expressed not only in leukocytes but also by chondrocytes (Horiuchi *et al.*, 2003). In CIA, granzyme A-deficient mice exhibited reduced joint damage, bone erosion and inflammatory cytokine production compared to wild-type controls. Interestingly, the authors also demonstrated that granzyme A induces osteoclastogenesis, which

may have relevance to its observed function in disease (Santiago *et al.*, 2017).

Mast cells are leukocytes with a role in immune surveillance and are considered some of the first cells responsible for engaging antigens or pathogens but are perhaps best known for playing a major role in allergic responses (Espinosa and Valitutti, 2017). Mast cell-resident serine proteinases include **tryptase** and **chymase**, which exhibit trypsin-like and chymotrypsin-like activities respectively (Caughey, 2016). In RA, both proteinases are present at the interface between cartilage and the invading pannus (Tetlow and Woolley, 1995) and can activate several proMMPs (Milner *et al.*, 2008). Tryptase can also cleave numerous ECM components (Milner *et al.*, 2008). However, administration of a tryptase inhibitor to mice following induction of arthritis by mBSA/**IL-1β** affected several parameters of inflammation but did not alter levels of joint destruction (Denadai-Souza *et al.*, 2017). Interestingly, detectable autoantibodies to tryptase in RA patients has led to it being proposed as a candidate autoantigen (Guo *et al.*, 2014). Tryptase is also a potent activator of PAR2 signalling and has been suggested to promote inflammatory arthritis *via* activation of this receptor (Palmer *et al.*, 2007).

Complement cascade

The complement cascade is an important component of innate immunity which upon activation has essential roles in pathogen clearance (Thurman *et al.*, 2017). Key components of this pathway are the serine proteinases **C1s**, C1r, C2, Factor B, Factor I and Factor D. A cascade of proteolytic events triggered by the detection of a foreign antigen results in the formation of a membrane attack complex (MAC) and cell lysis (for review, see Harris, 2018). Whilst a crucial component of normal host immune responses, dysregulated complement activation has been described in numerous inflammatory disorders, including RA. For example, all complement components are expressed in synovium, but levels are markedly elevated in RA (Gulati *et al.*, 1994). Chondrocytes are also capable of complement component expression, which can be modulated by pro-inflammatory cytokines (Bradley *et al.*, 1996). Active C1s has been localized to degrading articular cartilage from RA patients (Nakagawa *et al.*, 1999). Indeed, C1s has been shown to degrade collagen types I and II (Yamaguchi *et al.*, 1990), although it does not generate the 'three quarter and one quarter' fragments (Woolley *et al.*, 1975) following classical collagenase cleavage, suggesting that it is unlikely to be a major collagenase in arthritis. Cartilage matrix components released by proteolytic degradation can initiate complement activation (Happonen *et al.*, 2012), and targeting of the different factors within the complement cascade is now viewed as having therapeutic potential for RA (for an excellent review, see Thurman *et al.*, 2017).

Consistent with the paradigm that inflammation is important in OA, an essential role for the complement system in the pathogenesis of experimental OA has been reported (Wang *et al.*, 2011). Genomic and proteomic approaches demonstrated increased expression and activation of complement components in synovial fluid and membranes of patients with OA compared to control non-diseased tissue. Mice deficient in complement components C5 or C6 (not themselves serine proteinases, but downstream) exhibited protection against cartilage damage following meniscectomy or DMM. Conversely, mice deficient in CD59a, an inhibitor of MAC, had increased cartilage damage scores. Immunohistochemistry demonstrated the presence of MAC on the cell surface of human OA chondrocytes, and 'sub-lytic' levels of MAC were capable of inducing pro-inflammatory cytokines and matrix-degrading enzymes.

Activated protein C

Activated protein C (**APC**) is a serine proteinase activated from its precursor (protein C) by thrombin and has a well-established role as an antithrombotic, principally functioning to inactivate factors Xa and VIIIa in the regulation of blood coagulation pathways. However, it has also been demonstrated to have anti-apoptotic, anti-inflammatory and pro-regenerative functions (reviewed in Griffin *et al.*, 2015). APC has been detected in the synovial fluids from OA and RA patients (Buisson-Legendre *et al.*, 2004) and enhanced cytokine-induced cartilage breakdown which correlated with increased activation of **proMMP2** and proMMP9 (Jackson *et al.*, 2009). Moreover, MMP2 and APC co-localize in endothelial and synovial lining cells in RA joints (Buisson-Legendre *et al.*, 2004).

Interestingly, it has been suggested that APC can reduce the expression of MMP9 by binding to endothelial protein C receptor in synovial fibroblasts, a mechanism likely involving suppression of NFκB-mediated signalling. The same authors also demonstrated that APC increased the expression of MMP2 in these cells (Xue *et al.*, 2007). APC can induce cartilage breakdown in human OA cartilage even in the absence of pro-inflammatory cytokine stimulation, an effect dependent on metalloproteinase activity. Importantly, it was demonstrated that APC does not affect the expression of key metalloproteinases in OA chondrocytes (Jackson *et al.*, 2014a), and although unable to directly activate proMMP13 (Jackson *et al.*, 2009), it is likely that APC interacts with other metalloproteinases in the proteolytic cascades leading to cartilage destruction.

Proprotein convertases

The proprotein convertases subtilisin and kexin type (PCSK) are a family of nine calcium-dependent serine proteinases (PCSK1–9), which typically cleave after a highly basic sequence (RXR/KR↓), and are involved in the processing and activation of proteinases and growth factors. Several PCSKs, including the most extensively studied family member **furin** (PCSK3), contain transmembrane domains and are involved in the intracellular processing of substrates in the trans-golgi network (Milner *et al.*, 2008). Aberrant PCSK activities are involved in numerous disease states, and inhibitors are currently in clinical trials for cancer (Klein-Szanto and Bassi, 2017).

In an *ex vivo* model of cartilage breakdown using bovine cartilage, Dec-RVKK-CH_2Cl, an inhibitor of furin-like proteinases, provided protection against cytokine-induced collagen breakdown (Milner *et al.*, 2003). This inhibitor reduced levels of active collagenase in the conditioned medium, suggesting a role for these serine proteinases in collagenase activation pathways. Reduced levels of active MMP2 were also observed, the activation of which requires a complex mechanism involving TIMP2 and **MMP14**

(Milner *et al.*, 2003; Milner *et al.*, 2008). Furthermore, MMP14 can be processed by furin and has been previously shown to activate proMMP13, a major collagenase involved in the destruction of type II collagen (Knauper *et al.*, 1996; Sato *et al.*, 1996). Dec-RVKK-CH_2Cl also provided partial protection from proteoglycan breakdown, a process involving ADAMTS4 and ADAMTS5, both of which also contain furin-like activation motifs (Wang *et al.*, 2004; Longpre *et al.*, 2009). It is not yet clear which PCSK predominates in cartilage breakdown, although furin has been detected in cartilage and is elevated in OA (Moldovan *et al.*, 2000). In contrast, in murine CIA, administration of a furin inhibitor increased the arthritis score, joint destruction, MMP expression and activity within the joint whilst furin administration reduced these parameters (Lin *et al.*, 2012). Although the mechanism is undetermined, the authors hypothesized that furin may play an immunomodulatory role *via* mobilization of T_{reg} cells. Furthermore, it has recently been shown that gene silencing of furin increases the growth and invasiveness of human RA synoviocytes *in vitro*, as well as increasing their production of IL-1ß and TNFα (Wu *et al.*, 2017). Since RA is a disease with marked synovial inflammation, it is possible that furin may have differing roles in joint pathology depending on the type of cell and tissue that drives disease aetiology.

Paired amino acid cleaving enzyme 4 (PACE4; **PCSK6**) is a major PCSK involved in aggrecanase activation in human cartilage and is elevated in OA. Indeed, PACE4 silencing was able to reduce proteoglycan release and aggrecanase-generated neo-epitopes of aggrecan in cytokine-stimulated bovine and human OA cartilages (Malfait *et al.*, 2008). Interestingly, single-nucleotide polymorphisms in the PCSK6 gene have been linked to protection of human OA-related knee pain, whilst *pcsk6* null mice were significantly protected from pain in several algesiometric tests (Malfait *et al.*, 2012).

High temperature requirement proteinases

There are four high temperature requirement proteinases (HtrA1–4) which have important roles in protein quality control as well as the regulation of diverse biological processes such as cellular signalling (Tiaden and Richards, 2013). HtrA1 is the most studied in the context of arthritic disease; it has trypsin-like substrate specificity with an insulin-like growth factor binding protein domain and a kazal-like inhibitor domain at its N-terminus (Tiaden and Richards, 2013). A role for HtrA1 has been speculated in OA pathology for some time. An increase (~7-fold) in HtrA1 mRNA in OA cartilage compared with normal cartilage has been observed (Hu *et al.*, 1998), and protein levels have also been shown to be markedly increased in disease compared to age-matched controls (Chamberland *et al.*, 2009). Furthermore, HtrA1 levels are significantly elevated in OA synovial fluids (Grau *et al.*, 2006).

It remains unclear exactly how HtrA1 contributes to disease, although it has been linked to the inhibition of TGF-β signalling (Zurawa-Janicka *et al.*, 2010). Moreover, HtrA1 has the potential to degrade important ECM components, including fibronectin, type II collagen, decorin and aggrecan (Tiaden and Richards, 2013). Indeed, a novel cleavage site within the aggrecan interglobular domain has been identified, and neoepitope antibodies recognizing this site show higher reactivity in OA cartilage compared with normal controls (Chamberland *et al.*, 2009). HtrA1 can induce MMP1 and MMP3 in synovial fibroblasts by generating fibronectin fragments (Grau *et al.*, 2006), which may also contribute to OA through various mechanisms including cytokine production. It has been reported that upon disease initiation in the CIA model, HtrA1 expression is elevated, which correlates with resting chondrocytes undergoing terminal differentiation as well as an increase in joint swelling (Tsuchiya *et al.*, 2005). In a murine model of OA using a heterozygous mutant of COL2A1 – spondyloepiphyseal dysplasia congenita ($sedc/^{+}$) – which develops OA prematurely, cartilage immunostaining demonstrated increased HtrA1, MMP13 and discoidin-containing receptor-2 compared to control mice which preceded cartilage destruction, suggesting a potential role in disease initiation.

Proteinase-activated receptors

The PAR family have gained interest as potential therapeutic targets due to their critical roles in inflammation, vascular physiology, development and cancer progression. PARs offer an important link between extracellular proteinases and cellular responses because they are activated through a proteolytic mechanism, whereby proteinases cleave the N-terminus, exposing a tethered ligand domain. Once cleaved, the new N-terminus binds and activates the receptor (Yau *et al.*, 2013). Many of the PAR-activating serine proteinases are produced during tissue damage, and thus, PARs contribute to processes of repair and inflammation. Although there are four family members (PAR1–4), it is mainly PAR2 which has been associated with arthritis. PAR2 is activated by serine proteinases such as trypsin, tryptase or matriptase (Yau *et al.*, 2013). It is present in articular chondrocytes, with increased expression in osteoarthritic cartilage (Xiang *et al.*, 2006). Utilizing animal models, PAR2 has emerged as a target for preventing joint destruction. Confirming the important role PAR2 has as a pro-inflammatory receptor, a murine model of chronic inflammatory arthritis induced by Freund's complete adjuvant showed that the absence of PAR2 not only reduced joint inflammation but also protected cartilage integrity (Ferrell *et al.*, 2003). PAR2-deficient mice also showed protection from cartilage damage in the DMM model of OA (Ferrell *et al.*, 2010; Amiable *et al.*, 2011; Jackson *et al.*, 2014b), which has limited inflammation. Further studies on murine DMM indicated that PAR2 deficiency delayed osteophyte maturation (Huesa *et al.*, 2016), which reflects the previously observed delay in callus formation during bone fracture repair (Georgy *et al.*, 2012; O'Neill *et al.*, 2012). This suggests a role for PAR2 in driving pathogenic chondrocyte differentiation and/or maturation, separate from its pro-inflammatory action.

As a therapeutic target in musculoskeletal diseases, preventing PAR2 activation may be beneficial for cartilage repair or delaying OA progression, and the development of potent and specific PAR2 antagonists is ongoing (Yau *et al.*, 2013). A better understanding of the activation of PAR2 in different ECMs may provide new targets in the treatment of pathological joint destruction.

Endogenous serine proteinase inhibitors in matrix homeostasis

Like metalloproteinases, serine proteinases exist in a delicate equilibrium with cognate inhibitors. Serine proteinase inhibitors (serpins) are the largest family of endogenous serine protease inhibitors. A reactive centre loop (RCL) is positioned above the body of the serpin, acting as a 'bait region' for target proteinases. Upon cleavage, a covalent serpin-proteinase complex forms, concomitant with a conformational change rendering the proteinase inactive (Huntington, 2011).

One of the most studied members of the serpin family, **SERPINA1** (α1-antitrypsin), has been identified as a differentiation marker in chondrogenesis (Boeuf *et al.*, 2008). This serpin is up-regulated during chondrogenic induction from bone marrow-derived stem cells and down-regulated during chondrocyte dedifferentiation in monolayer culture. The expression of SERPINA1, as well as other serpins including SERPINE1 (plasminogen activator inhibitor 1) and SERPINE2 (protease nexin 1) is up-regulated in chondrocytes by cytokine-stimulation (Treadwell *et al.*, 1991; Fischer *et al.*, 1999; Santoro *et al.*, 2015), perhaps suggesting an important protective mechanism for articular chondrocytes to prevent cartilage damage by counteracting inflammation-induced proteinase activities. Indeed, some studies have assessed serpins in synovial fluid, with levels significantly increased in RA and OA, compared to controls (Belcher *et al.*, 1996; Maciejewska-Rodrigues *et al.*, 2010). *In vivo*, SERPINA1 administration delayed onset and reduced disease scores in the inflammatory CIA model whilst, *ex vivo*, SERPINA1 afforded partial protection from cytokine-stimulated breakdown in bovine cartilage (Milner *et al.*, 2001; Grimstein *et al.*, 2011). As each serpin inhibits its own spectrum of target proteinases, the partial protection from breakdown suggests that different serine proteinases may be involved. In normal human cartilage, basal levels of SERPINA1 and SERPINA3 (α1-antichymotrypsin) expression have been reported, with reduced expression of SERPINA1 in OA cartilage (Boeuf *et al.*, 2008). Interestingly, active MMPs have been shown to inactivate serpins by cleavage within the RCL (Mast *et al.*, 1991; Lijnen *et al.*, 2000; Lijnen *et al.*, 2001), perhaps indicating that MMP activity could further enhance cartilage destruction indirectly, by increasing the proteolytic burden from serine proteinases.

Gaining a detailed understanding of serine proteinase inhibitor function in cartilage will not only provide insight into which inhibitors are essential for chondroprotection but will also likely highlight key serine proteinases involved in ECM catabolism.

Targeting of serine proteinases in arthritis – potentials and limitations

In OA, there are no therapies that alter disease progression, and the majority of current treatments involve pain management, followed ultimately by joint replacement. Although disease-modifying treatments for RA, such as biologics targeting TNFα, are effective for many patients, some do not respond to these therapies (Kiely *et al.*, 2012). Thus, there is clearly a requirement to develop new anti-arthritic drugs to help address dysfunctional immunoregulation and/or prevent or perturb the loss of cartilage from the joint surface.

The failure of clinically effective MMP inhibitors has led to a degree of scepticism regarding proteinases as relevant targets for therapeutic intervention. Despite a new drive for specific MMP inhibitors in recent years, including some marked advances in the development of MMP13 inhibitors (recently reviewed in Xie *et al.*, 2017), the active site architecture of several MMPs is similar, with substrate specificity often determined by the non-catalytic haemopexin domain (Rowan *et al.*, 2008). Whilst we concur ECM turnover in arthritis is likely predominantly metalloproteinase-driven, the critical importance of serine proteinase activities in regulating MMP activities at different levels is clear and may represent novel therapeutic opportunities. The emergence of genomic and proteomic advances over the past two decades has transformed our understanding of serine proteinase involvement and indeed identified new proteinases, which may play fundamental roles in the pathology underlying arthritic diseases. A summary of the key proteinases outlined in this review is depicted in Figure 1.

Serine proteinases have been targets for therapeutic intervention for some time. Perhaps the most notable success story in recent years has been **coagulation factor X** (factor Xa) inhibitors which were developed following a flood of interest into more satisfactory anti-thrombotic agents than the widely used **warfarin** and vitamin K (Yeh *et al.*, 2012). Like many trypsin-like serine proteinases, coagulation factor X exhibits a hydrophilic active site cleft, with an aspartate residue in the S1 pocket providing a strong requirement for basicity in the P1 position. Hence, the design of orally bioavailable drugs represented a challenge, although not an insurmountable one (Quan *et al.*, 2003), and such compounds were eventually licensed for use as anti-coagulants in numerous disorders (Yeh *et al.*, 2012). Other serine proteinases do not have such subsite binding preferences; indeed, inhibitors of NE have been designed, with one drug, **sivelestat**, becoming licensed for the treatment of acute lung injury in Japan and South Korea, although its clinical efficacy is controversial (Aikawa and Kawasaki, 2014). In a further example, SERPINA1 is routinely administered to patients suffering from α1-antitrypsin deficiency, a common genetic disorder, which manifests as emphysema and impaired liver function (Teschler, 2015).

If serine proteinases are to be considered potential targets for therapeutic intervention for attenuating joint destruction in arthritis, the following questions must be answered: (i) What is the best target and can it be selectively inhibited? (ii) Is the target disease-specific and are there likely to be differences amongst patients diagnosed with the same disease? (iii) When would a therapeutic be administered and what would be the most appropriate route of administration?

The most tractable serine proteinase targets will likely differ between RA and OA. As an autoimmune disorder with significant inflammatory infiltrate, it is likely that cartilage ECM destruction in RA is driven by proteinases from the hyperplastic synovial membrane or degranulation of infiltrating leukocytes. Perhaps the most exciting targets for RA would include NSPs, complement or mast cell-derived proteinases. In contrast, cartilage degradation in OA is probably driven by

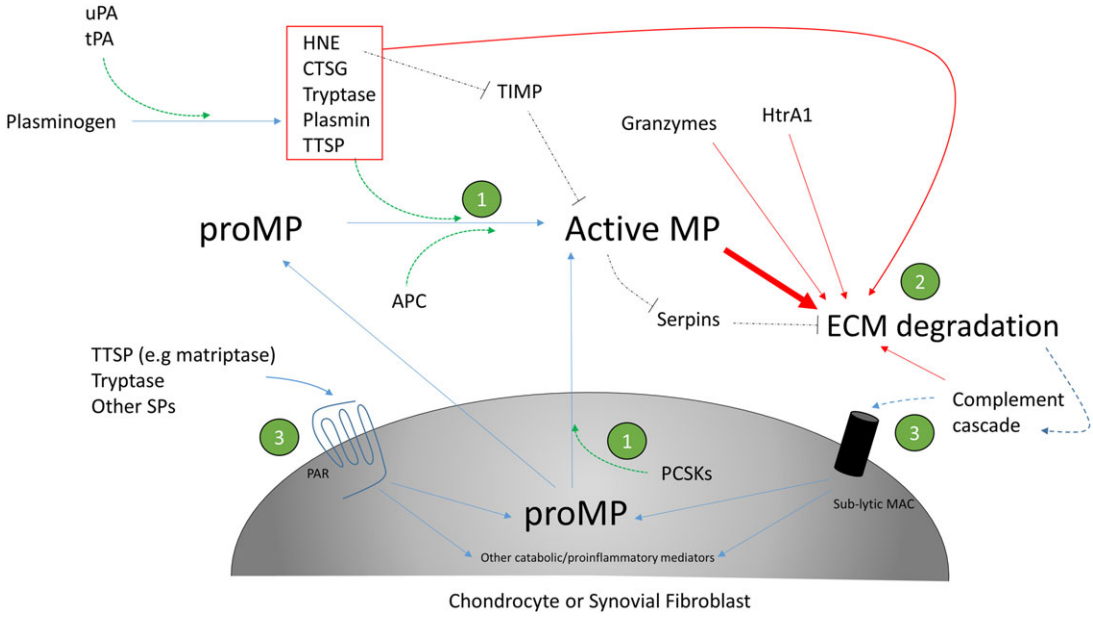

Figure 1

Summary schematic for key mechanisms of cartilage destruction involving serine proteinases. In arthritis, destruction of the cartilage ECM is predominately due to the action of metalloproteinases (MP) such as MMP and ADAMTS proteinases. However, serine proteinases play key roles in the regulation of catabolic process, such as activation of proenzymes (1). Direct proteolysis of the ECM by serine proteinases such as plasmin, immune cell-derived serine proteinases, complement and HtrA1 also likely contribute to pathology (2). Serine proteinases may also induce the expression of metalloproteinases through PAR and complement activation, for example (3).

chondrocyte-derived proteinases, although mediators from an inflamed synovium should not be excluded. TTSPs such as matriptase, or the serine proteinase receptor, PAR2, could represent exciting targets to limit MMP-dependent cartilage destruction. A wealth of data suggest that HtrA1 likely has an important role in OA although further work is required to establish the central mechanism by which it may contribute.

A key hurdle is the heterogeneity of disease for both RA and OA. Differences in disease aetiologies between individuals suggest that a 'one-size-fits-all' approach is unlikely to be successful, and a more tailored approach is required. Exciting advances have been made in the detection of *active* proteinases within biological samples using probes which are specific for a particular proteinase (reviewed in Sanman and Bogyo, 2014). Profiling of proteinases using activity-based probes in arthritis could reveal which proteinases are most active within synovial fluid, for example, and specific therapeutics administered to individual patients based on the identified profile.

Conclusions

Although our understanding of the roles of serine proteinases in cartilage ECM turnover in arthritis has improved dramatically over the last two decades, details of the biology underpinning disease remain incomplete, still more questions than answers. Previous studies suggest serine proteinases are amenable to therapeutic intervention with sufficient structural differences to allow for selective targeting. Findings from *in vivo* models of arthritis do not always translate to the human condition, but if they do for specific serine proteinases, this could prove an attractive strategy to limit the destruction of the cartilage ECM.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017a,b).

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Conflict of interest

The authors declare no conflicts of interest.

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